Dietary Calcium Chloride vs. Calcium Carbonate Reduces Urinary pH and Phosphorus Concentration, Improves Bone Mineralization and Depresses Kidney Calcium Level in Cats[1,2]

F.J.H. PASTOOR,*[3] R. OPITZ,* A. TH. VAN 'T KLOOSTER† AND A. C. BEYNEN*†

*Department of Laboratory Animal Science and †Department of Large Animal Medicine and Nutrition, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

ABSTRACT The effect of dietary calcium chloride vs. calcium carbonate on mineral metabolism was studied in cats. Ovariectomized cats and female kittens were fed purified diets with a normal calcium level (9.5 mmol Ca/MJ) but containing either calcium carbonate or calcium chloride, or were fed diets with a high calcium level (17.7 mmol Ca/MJ) containing either calcium carbonate alone or equimolar amounts of both calcium carbonate and calcium chloride. A 4 × 4-wk cross-over study using adult cats and a 31-wk parallel study using kittens were conducted. Calcium, phosphorus and magnesium balances were established regularly. In the course of the experiment with the kittens, blood samples were taken and X-ray photographs of the tibiae made. At the age of 39 wk, the kittens were killed, and organs and bones were collected. In both adult cats and kittens fed the high calcium diets, urinary concentrations of magnesium and phosphorus and apparent absorption of these minerals were lower than after feeding the normal calcium diets. Urinary pH and phosphorus concentration were lower in cats and kittens fed diets with calcium chloride instead of calcium carbonate. Body weight gain and tibia growth in the kittens tended to be greater after feeding the diets with calcium chloride. Calcium chloride vs. calcium carbonate and also supplemental calcium chloride in the high calcium diet significantly stimulated femur density and reduced renal calcium concentration. J. Nutr. 124: 2212–2222, 1994.

INDEXING KEY WORDS:

• mineral excretion • bone mineralization
• calcium chloride • nephrocalcinosis • cats

Urolithiasis is a common disorder in cats. Almost 85% of feline uroliths are composed of struvite (magnesium-ammonium-phosphate-hexahydrate) (Osborne et al. 1985). Spontaneous precipitation of struvite does not occur when the product of urinary concentrations of its components is below the formation product. The product of the three constituent ion concentrations is diminished by lowering urinary pH (Buffington et al. 1989). Indeed, reduction of urinary pH to values of 5.7–5.9 has been shown to prevent struvite urolithiasis in cats (Buffington et al. 1985, Taton et al. 1984). Ammonium chloride is frequently used for urinary acidification to prevent urolithiasis in cats (Taton et al. 1984). However, ammonium chloride treatment of cats may be associated with enhanced urinary excretion of calcium (Ching et al. 1989). This also occurs in rats and is accompanied by loss of bone minerals (Kraut et al. 1986, Kunkel et al. 1986).

Substitution of calcium chloride for calcium carbonate in the diet is anticipated to lower urinary pH in cats (Izquierdo and Czarnecki-Maulden 1991, Kienzle et al. 1991). Calcium chloride may thus be considered as an alternative to ammonium chloride in the prevention of struvite urolithiasis. Replacement of dietary calcium carbonate by calcium chloride raises urinary calcium excretion in rats (Schaafsma et al. 1985, Whiting and Cole 1986), but it was not known whether this extends to cats. The extra intake of calcium in the form of calcium chloride could have additional advantages with regard to prevention of struvite urolithiasis; apart from urinary acidification (Izquierdo and Czarnecki-Maulden 1991, Kienzle et al. 1991), the extra calcium intake per se may reduce urinary concentrations of the struvite constituents

[1]F.J.H. Pastoor was supported by Rodi B.V., Opmeer, The Netherlands.

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[3]To whom correspondence should be addressed.

0022-3166/94 $3.00
Manuscript received 15 October 1993. Initial review completed 13 December 1993. Revision accepted 5 May 1994.

magnesium and phosphorus in cats (Pastoor et al. 1994).

It was not known whether extra dietary calcium chloride or equimolar replacement of calcium carbonate by calcium chloride influences urinary mineral excretion and bone density in cats. Thus, we measured mineral excretion in adult cats fed diets containing either calcium carbonate or calcium chloride. In an attempt to augment the effects of calcium chloride, diets with either a normal (9.5 mmol Ca/MJ) or high calcium level (17.7 mmol/MJ) were used. The effects of calcium chloride on growth and bone mineralization were studied in female kittens. Because calcium chloride reduces the degree of kidney calcification in rats (unpublished results) compared with calcium carbonate the degree of renal mineralization was also determined in the kittens.

MATERIALS AND METHODS

The protocols of the experiments were approved by the animal experiments committee of the Department of Laboratory Animal Science.

Experiment 1

Animals. Eight specified pathogen free-derived, ovariectomized cats (n = 5, Ico:FecEur(Tif), Iffa Credo, L'Arbresle, France; n = 3, Hsd/Cpb:CaDs, Harlan Cpb, Zeist, The Netherlands) were used. At the start of the experiment the cats were ~3 y of age. The cats were ovariectomized for two reasons: 1) when intact female cats are in heat, they often refuse to eat, which would interfere with the execution of a dietary balance study and 2) adult female cats kept as pets are often neutered, thus, by using ovariectomized cats the outcome of the study may have greater practical value.

Housing and diets. The cats were housed as a group in a room (2.2 × 4.5 × 3.0 m) with eight open stainless steel cages (116 × 56 × 67 cm), in which a controlled light cycle (light: 0700–1900 h), temperature (20–23°C) and humidity (50–65%) were maintained. During the acclimatization period of 1 mo, the cats were fed a purified diet, which was formulated according to the minimum requirements of cats (National Research Council 1986) and calculated to contain 9.5 mmol calcium/MJ (normal calcium level), mainly in the form of calcium carbonate (Table 1).

TABLE 1

***Composition of the experimental diets*[1,2]**

	Normal Ca		High Ca	
	CO_3^{2-}	Cl^-	CO_3^{2-}	CO_3^{2-} + Cl^-
Ingredient, *g/kg*				
$CaCl_2$	0	17.851	0	17.851
$CaCO_3$	16.155	0	32.310	16.155
Dextrin	337.632	335.936	321.477	319.781
Constant components[3]	646.213	646.213	646.213	646.213
Chemical analysis,[4] *mmol/kg*				
Calcium	181/167	170/168	325	316/308
Phosphorus	163/164	168/167	168	168/158
Magnesium	15/14	15/14	16	15/15

[1] The metabolizable energy density of the diets was calculated to be 19.7 MJ/kg, using values of 16.8, 16.8 and 37.8 kJ/g for metabolizable energy content of protein, carbohydrates and fat respectively.

[2] Calculated dietary calcium concentrations: Normal Ca: 9.5 mmol/MJ; High Ca: 17.7 mmol/MJ.

[3] The constant components consisted of the following (g): egg white, 186.5; herring meal, 56.2; beef tallow, 197.2; corn oil, 8.5; glucose, 56.2; cooked cornstarch, 56.2; cellulose, 11.2; $NaH_2PO_4 \cdot 2H_2O$, 21.883; $MgCO_3$, 0.67; Na_2CO_3, 19.28; taurine, 0.38; vitamin premix, 12; mineral premix, 20. The diets were formulated taking into account analyzed calcium, phosphorus and magnesium concentrations in the egg white and herring meal preparations. These concentrations were as follows (mmol/kg product): egg white; calcium, 12.97; magnesium, 18.92; phosphorus, 20.99; herring meal; calcium, 426.65; magnesium, 74.04; phosphorus, 645.79. The vitamin premix consisted of (mg/12 g): retinyl acetate and retinyl palmitate (150 μg/mg), 6.3; cholecalciferol (12.5 μg/mg), 0.94; all-rac-α-tocopheryl acetate (0.5 mg/mg), 56.6; menadione, 0.094; thiamin, 4.7; riboflavin, 3.78; pyridoxine, 3.78; nicotinamide, 37.8; DL-calcium pantothenate (0.45 mg/mg), 10.48; pteroylmonoglutamic acid, 0.755; biotin, 3.0; cyanocobalamin (1 μg/mg), 18.9; choline chloride (0.5 mg/mg), 5228.46; myo-inositol, 200; cooked cornstarch 6424.411. The mineral premix consisted of (mg/20 g): KCl, 7191; $FeSO_4 \cdot 7H_2O$, 375.6; $CuSO_4 \cdot 5H_2O$, 18.5; MnO_2, 7.4; $ZnCl_2$, 98.3; KI, 0.45; $Na_2SeO_3 \cdot 5H_2O$, 0.31; cooked cornstarch, 12308.44.

[4] Figures before slash: Experiment 1; figures after slash: mean values (four batches of feed) for Experiment 2. The high calcium carbonate diet was not used in Experiment 2.

The cats were given free access to the diet for 2 h (0900–1100 h) per day. During the feeding period each cat was confined to its own cage. Demineralized water was always freely available.

After the acclimatization period, the cats were fed the four experimental diets (Table 1), including the pre-experimental diet, according to a balanced Latin square design. The diets had either a normal (9.5 mmol/MJ) or high calcium level (17.7 mmol/MJ). The normal calcium diets contained either calcium chloride or an equimolar amount of calcium carbonate. The high calcium diets were formulated by addition of calcium carbonate to the normal calcium diets and contained either calcium carbonate alone or equimolar amounts of both calcium carbonate and calcium chloride. The ingredient and analyzed composition of the diets is given in Table 1.

Collection of samples. Each dietary period lasted 4 wk. During the last 7 d of each period, the cats were confined individually in their cages. Feed intake was recorded, and urine and feces were collected. The method to collect excreta of cats has been published (Pastoor et al. 1990). At the end of each period the cats, after having been deprived of food for 22 h, were anesthetized (0.1 mg medetomidine/kg body wt, intramuscularly) and blood samples were taken from the jugular vein into heparinized tubes. Immediately after blood sampling, an antidote (0.5 mg atipamezole/kg body wt, intramuscularly) was given.

Experiment 2

Animals. Twenty-four 8-wk-old, weanling cats (n = 21, Hsd/Cpb:CaDs, Harlan Cpb, Zeist, The Netherlands; n = 3, Fec:Kun, Catholic University Nijmegen, Nijmegen, The Netherlands) were used.

Housing and diets. Three groups of eight kittens each were stratified for body weight and litter and housed in separate stalls (2.2 × 2.6 × 3.0 m) in the same room (8 × 6 × 3 m). Each stall had four open stainless steel cages (116 × 56 × 67 cm). During the balance periods four extra cages were placed in each stall. In the room, lighting (light: 0700–1900 h), temperature (18–23°C) and humidity (50–70%) were controlled.

On arrival, the kittens had free access to a commercial diet (Cat Diet LF-32, Hope Farms, Woerden, The Netherlands) and tap water. They were gradually transferred, over 3 d, to either the normal calcium diets or the high calcium diet containing calcium carbonate plus calcium chloride (Table 1). The kittens were given free access to the purified diets and demineralized water. Body weight of the kittens was measured weekly.

Collection of samples. Balance studies were performed on cats at the ages of 15, 21, 31 and 39 wk. During periods of 6 d each, the cats were housed individually. They were allowed to leave their cages for 1 h/d. Food intake was recorded and urine and feces were collected each day.

At the age of 11 wk and at the end of each balance period the cats were anesthetized (0.1 mg atropine, 15 mg ketamine, and 0.5 mg xylazine/kg body wt, intramuscularly). Blood was taken from the jugular vein and samples collected in heparinized tubes. An X-ray photograph (MCD 125, Philips, Eindhoven, The Netherlands) of the tibia of each cat was made. A leaden ruler was photographed simultaneously to determine bone length on the X-ray photographs.

Immediately after blood sampling at the age of 39 wk, the anesthetized cats were killed by an overdose of sodium pentobarbital (0.4–0.8 g/cat, administered intravenously). Kidneys, heart, liver and left femur and tibia were removed. Kidney capsules were discarded. The organs were weighed and frozen at –20°C until chemical analysis.

Interventions in the course of the experiment. In the first week, the kittens were found to have coccidiosis. They were treated for 9 d with sulfamethoxypyridazin (50 mg/kg on d 1 and 7; 25 mg/kg on d 2, 3, 8 and 9) but remained coccidiosis positive. We then administered 25 mg toltrazuril/L drinking water for two consecutive days per week during wk 3–6 of the experiment. After wk 3, the kittens fed the calcium chloride containing diets were free from coccidiosis and after wk 4 oocysts of *Coccidia* were absent in the feces of the other kittens as well.

Two kittens, that were fed the normal calcium diet with calcium carbonate had to be removed in wk 8 of the experiment, because they did not accept the purified diet. After transfer to a commercial cat diet they rapidly attained a good condition.

Preparation of samples. Feces, urine and plasma samples were prepared for analysis as described (Pastoor et al. 1994).

Organs were homogenized in demineralized water with an Ultra-turrax (TP 18/10, Janke & Kunkel, Staufen im Breisgau, Germany). Homogenized organ samples were dried and ashed as described for feces.

Femur and tibia were wrapped in tinfoil and heated to 121°C in a pressure cooker for 10 min at a pressure of 1.0 kg/cm^2. Femurs and tibiae were cleaned of adhering matter and their length and circumference were measured. Femurs were sawn transversely into two halves and bone marrow was removed. Femur volume was determined by weighing in air and under water. Then, femurs were dried and ashed as described for feces.

Chemical analyses. Calcium, magnesium and phosphorus in feed, feces, urine, plasma, organs and femurs were analyzed as described (Pastoor et al. 1994). In Experiment 2, hydroxyproline in nonacidified urine was determined using the Hypronosticon test-combination kit (Organon Teknika, Boxtel, The Netherlands). In Experiment 1, net urinary acid excretion [urinary titratable acid – bicarbonate + ammonium] was determined by a titrimetric method

(Chan 1972) using a semi-automatic titrator (TTT 80 titrator and ABU 80 autoburette, Radiometer, Copenhagen, Denmark). Urinary pH was measured with an electrode (Phm 83 autocal pH meter, Radiometer). We had found that the pH of freshly voided urine increases while in the litter box during the day, and thus we corrected the pH of 24-h urine samples using a regression line, $Y = 3.238 + 0.534X$ ($r = 0.91$, $P < 0.001$, $n = 20$), established with urine samples for which the pH was measured immediately after micture (Y) and 16–24 h later (X). The range of the X values was 7.0–9.6, which corresponds with that for the urinary pH values measured in the present studies.

For all chemical analyses accuracy was verified to be within 5% deviation from the targets with reference samples (reference serum, Roche N, Roche Diagnostica and in-house reference pools of feed, feces and urine).

Statistical analyses

All statistical analyses were performed according to Steel and Torrie (1981), using a SPSS/PC+ computer program (SPSS 1988a and 1988b). The two-sided level of statistical significance was pre-set at $P < 0.05$.

In Experiment 1, multivariate analyses of variance (MANOVA) was performed with cat, time, calcium level, calcium source and calcium level × source interaction. Homogeneity of variance was checked (Barlett's test). In fact, the dietary calcium level in the two high calcium diets was accommodated by addition of calcium carbonate to the two normal calcium diets. Thus, when MANOVA yielded a significant effect of calcium level, this referred to supplemental calcium carbonate. Nevertheless, the effect of extra calcium carbonate as compared with calcium chloride, when added to the normal calcium diet with calcium carbonate, can be compared. Significant effects of diet were identified by multiple comparisons (contrasts with level of statistical significance pre-set at $P < 0.0125$ according to Bonferroni's adaptation) in a one-way ANOVA, using the residual sum of squares of the MANOVA.

In Experiment 2, MANOVA (repeated measures) was used to evaluate effects of age, diet and their interaction. Group means at the same age were compared by one-way ANOVA followed by the Tukey test or, for non-normally distributed data, by the Kruskal-Wallis test followed by Mann-Whitney U tests (with level of statistical significance pre-set at $P < 0.017$ according to Bonferroni's adaptation).

RESULTS

Experiment 1

Food intake and body weight. Food intake of the adult cats was not influenced by dietary composition (Table 2). Body weights were slightly, but significantly higher after feeding the calcium chloride containing diets.

Mineral balance. Retention of minerals was calculated as intake minus urinary plus fecal excretion and expressed as millimoles per day (Table 2). When fed the high calcium diets, cats had greater fecal excretion of calcium than when fed the low calcium diets. Feeding the diets with calcium chloride depressed urinary excretion and raised retention of calcium. Urinary excretion of magnesium was lower and fecal excretion of magnesium was higher when the cats were fed the high calcium diets. Retention of magnesium was not affected by dietary composition. Urinary excretion of phosphorus was affected by source and level of calcium and its interaction. High calcium intakes and calcium chloride as compared with calcium carbonate depressed urinary phosphorus excretion. Fecal excretion of phosphorus rose after high calcium intakes. Retention of phosphorus was higher when the cats were fed the diets containing calcium chloride instead of calcium carbonate.

Mineral absorption. Apparent absorption was calculated as intake minus fecal excretion and expressed as percentage of intake. Percentages of apparent absorption of calcium showed relatively high within-diet variation and did not significantly differ between dietary treatments. Absorptions of magnesium and phosphorus were lower at high rather than normal calcium intakes (**Fig. 1**). The type of anion did not significantly affect magnesium and phosphorus absorption.

Urinary composition. Urinary volume was slightly higher when the cats were fed the calcium chloride diets (Table 2). Urinary pH reached high values when the calcium carbonate diets were fed and low when calcium chloride was substituted for calcium carbonate (**Fig. 2**). Urinary concentrations of magnesium and phosphorus were lower with high calcium intakes. Urinary concentrations of phosphorus and calcium were lower when calcium carbonate was replaced by calcium chloride.

Urinary acid excretion. Urinary excretion of titratable acid and ammonium, and consequently also that of net acid, were higher when the cats were fed the calcium chloride diets instead of the calcium carbonate diets (Table 2). When the dietary calcium level was raised by addition of calcium carbonate excretion of titratable acid was lower.

Plasma minerals. Plasma calcium, magnesium and phosphorus concentrations were not affected by dietary composition; average concentrations were 2.36

TABLE 2

Experiment 1: Food intake, body weight, urinary volume and acid excretion, and mineral balance in adult cats fed the experimental diets[1,2,3]

	Normal Ca		High Ca		
	CO_3^{2-}	Cl^-	CO_3^{2-}	CO_3^{2-} + Cl^-	Significance[4]
Food intake, *g/d*	42.2 ± 2.3	43.8 ± 3.0	44.2 ± 3.0	47.3 ± 3.7	
Body weight, *kg*	3.05 ± 0.15	3.15 ± 0.14	3.01 ± 0.11	3.09 ± 0.13	Cl
Urinary volume, *mL/d*	66.1 ± 6.4	74.0 ± 7.3	68.0 ± 7.5	76.9 ± 7.7	Cl
Urinary acid excretion, *mmol/d*					
Titratable acid	−7.8 ± 0.5	−1.2 ± 0.5[C]	−9.9 ± 0.5[A]	−3.4 ± 0.5[B,D]	Ca, Cl
Ammonium	1.1 ± 0.1	2.3 ± 0.2[C]	1.3 ± 0.2	2.0 ± 0.2[D]	Cl, Ca·Cl
Net acid	−6.7 ± 0.4	1.1 ± 0.4[C]	−8.6 ± 0.4[A]	−1.4 ± 0.4[B,D]	Ca, Cl
Balance, *mmol/d*					
Calcium					
Intake	7.6 ± 0.4	7.4 ± 0.5	14.3 ± 1.0[A]	15.0 ± 1.2[B]	Ca
Urinary output	0.05 ± 0.01	0.04 ± 0.01	0.07 ± 0.01	0.05 ± 0.01[D]	Ca, Cl
Fecal output	7.4 ± 0.5	6.7 ± 0.5	15.2 ± 0.9[A]	13.7 ± 1.3[B]	Ca
Retention	0.2 ± 0.7	0.7 ± 0.5	−1.0 ± 0.5	1.3 ± 0.9	Cl
Magnesium					
Intake	0.64 ± 0.03	0.63 ± 0.04	0.71 ± 0.05	0.72 ± 0.06[B]	Ca
Urinary output	0.20 ± 0.02	0.20 ± 0.02	0.17 ± 0.02	0.16 ± 0.02	Ca
Fecal output	0.44 ± 0.02	0.41 ± 0.04	0.58 ± 0.03[A]	0.52 ± 0.04	Ca
Retention	0.01 ± 0.05	0.02 ± 0.03	−0.04 ± 0.03	0.03 ± 0.04	
Phosphorus					
Intake	6.8 ± 0.4	7.4 ± 0.5	7.4 ± 0.5	8.0 ± 0.6	Ca, Cl
Urinary output	4.4 ± 0.3	4.2 ± 0.3	3.7 ± 0.4[A]	2.7 ± 0.3[B,D]	Ca, Cl, Ca·Cl
Fecal output	2.5 ± 0.2	2.6 ± 0.3	3.7 ± 0.3[A]	4.4 ± 0.4[B]	Ca
Retention	−0.1 ± 0.2	0.5 ± 0.2	−0.0 ± 0.2	0.8 ± 0.3[D]	Cl

[1]Values are means ± SEM (n = 8).

[2]Contrast significance ($P < 0.0125$): A, effect of calcium level with calcium carbonate as calcium source; B, effect of calcium level with calcium chloride in the diet; C, effect of calcium chloride vs. carbonate in normal calcium diets; D, effect of calcium chloride vs. carbonate in high calcium diet.

[3]Calculated dietary calcium concentrations: Normal Ca: 9.5 mmol Ca/MJ; High Ca: 17.7 mmol Ca/MJ.

[4]Significance: Multivariate ANOVA ($P < 0.05$); Ca = effect of calcium level (high vs. normal calcium diets); Cl = effect of anion (diets with calcium chloride vs. diets with calcium carbonate); Ca·Cl = interaction of calcium level and type of anion.

± 0.04, 0.80 ± 0.01 and 1.01 ± 0.04 mmol/L, respectively (means ± SEM, n = 8).

Plasma activity of alkaline phosphatase. The activity of alkaline phosphatase in plasma was slightly, but significantly lower in cats fed dietary calcium chloride vs. calcium carbonate; the values were 0.90 ± 0.21 and 0.83 ± 0.16 μkat/L for the normal calcium carbonate and chloride diets, and 0.87 ± 0.18 and 0.79 ± 0.18 μkat/L for the high calcium carbonate and chloride diets (means ± SEM, n = 8, MANOVA: $P < 0.05$).

Urea and creatinine levels. Urinary urea excretion and plasma concentrations of urea and creatinine were not affected by dietary composition; average values were 10.9 ± 0.4 mmol/(d·kg body wt), 6.9 ± 0.3 mmol/L and 133 ± 7 μmol/L (means ± SEM, n = 8). Urinary creatinine excretion was slightly, but significantly higher when the chloride diets were fed; the values were 328 ± 18 and 356 ± 18 μmol/(d·kg body wt) for cats fed the normal calcium carbonate and chloride diets and 329 ± 13 and 336 ± 18 μmol/(d·kg body wt) for those fed the high calcium carbonate and chloride diets (means ± SEM, n = 8, MANOVA: $P < 0.05$).

Experiment 2

Body weight and food intake. During the experiment with kittens, body weights of the three dietary groups were not significantly different, but group mean body weights of the kittens fed the normal and high calcium diets containing calcium chloride were systematically higher (Fig. 3). Food intake was similar for all three dietary groups (Table 3).

Mineral balance. Urinary output of calcium and magnesium were not significantly affected by dietary composition (Table 3). Urinary output of phosphorus was lower in the kittens fed the high calcium diet. Fecal excretion of calcium, magnesium and phosphorus were higher in the kittens fed the high

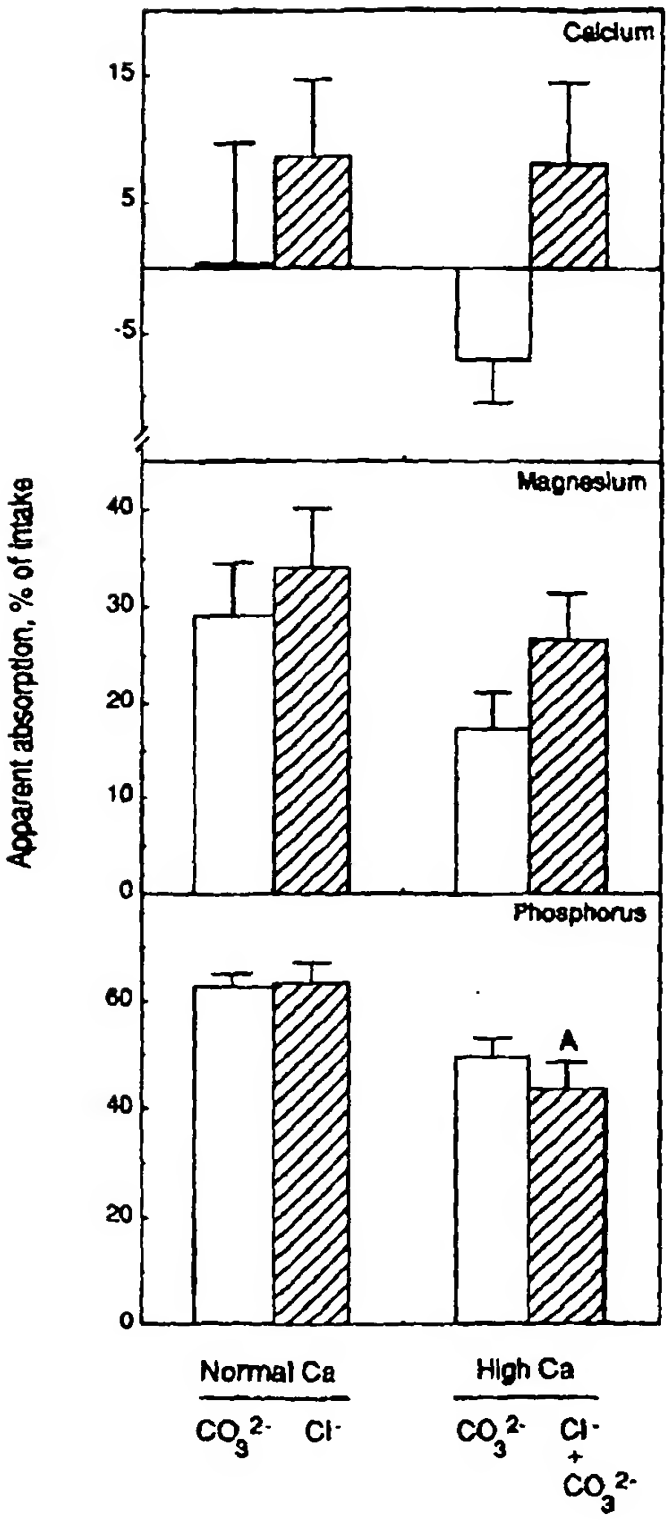

FIGURE 1 Experiment 1: Apparent absorption of calcium, magnesium and phosphorus in adult, ovariectomized cats fed diets containing either normal or high levels of calcium using either calcium carbonate, calcium chloride or a combination (Table 1). Mineral absorption was calculated as mineral intake minus fecal excretion, and expressed as percentage of intake. Results are expressed as means ± SEM (n = 8). Multivariate ANOVA yielded significant effects ($P < 0.05$) of dietary calcium level on the absorption of magnesium and phosphorus. Contrast significance ($P < 0.0125$): *A*, effect of calcium level with calcium chloride in the diet.

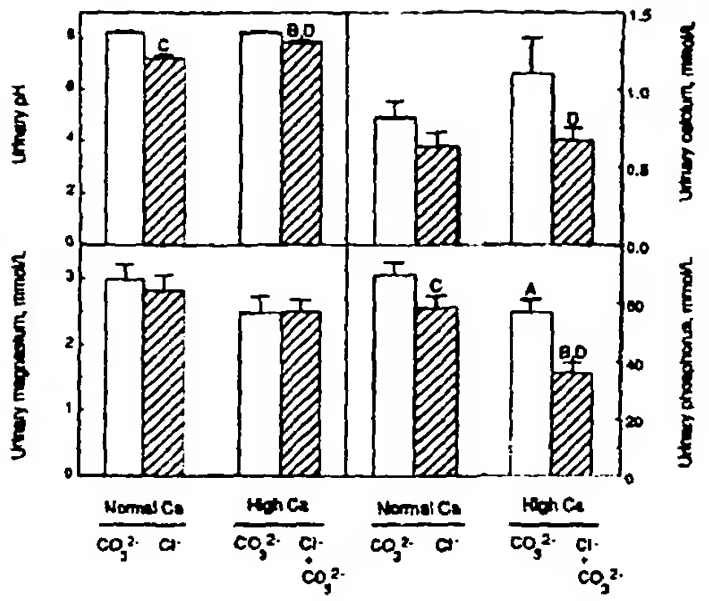

FIGURE 2 Experiment 1: Urinary pH and urinary concentrations of calcium, magnesium and phosphorus in adult, ovariectomized cats fed diets containing either normal or high levels of calcium using either calcium carbonate, calcium chloride or a combination (Table 1). Results are expressed as means ± SEM (n = 8). Multivariate ANOVA yielded significant effects ($P < 0.05$) of dietary anion type, calcium level and its interaction on urinary pH and urinary phosphorus concentration. Urinary concentration of calcium was affected by anion type, and urinary concentration of magnesium was influenced by dietary calcium level. Contrast significance ($P < 0.0125$): *A*, effect of calcium level for diets with calcium carbonate as calcium source; *B*, effect of calcium level for diets with calcium chloride; *C*, effect of calcium chloride vs. carbonate in normal calcium diets; *D*, effect of calcium chloride vs. carbonate in high calcium diets.

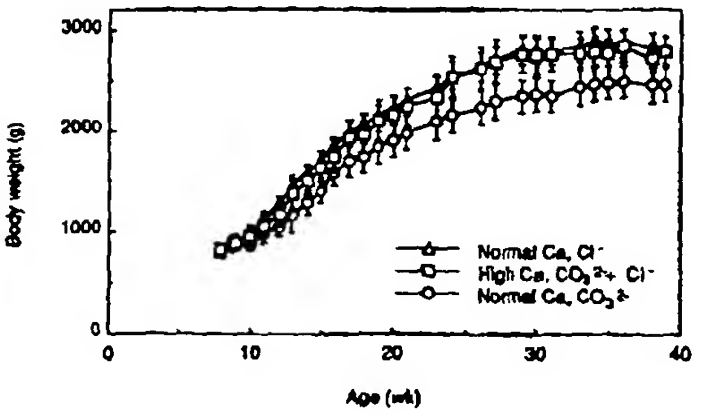

FIGURE 3 Experiment 2: Time course of body weight of female kittens fed diets containing either normal levels of calcium with either calcium carbonate or calcium chloride or a high level of calcium with a combination of the two salts (Table 1). Results are expressed as means ± SEM (normal calcium diet with calcium carbonate: n = 6; normal and high calcium diet with calcium chloride: n = 8). One-way ANOVA was performed to compare the three dietary groups at the same age: P = 0.13–0.87.

TABLE 3

Experiment 2: Food intake, urinary volume and balance of calcium, magnesium and phosphorus in female kittens fed diets containing normal calcium diets with either calcium carbonate or calcium chloride or a high calcium diet with both calcium salts[1,2]

	Age, *wk*				
	15	21	31	39	Significance[3]
Food intake, *g/d*					A
Normal Ca, CO_3^{2-}	57.0 ± 5.9	58.8 ± 6.3	50.4 ± 5.1	43.4 ± 5.7	
Normal Ca, Cl^-	65.7 ± 3.0	63.1 ± 3.8	51.1 ± 5.2	44.3 ± 5.0	
High Ca, CO_3^{2-} + Cl^-	64.1 ± 7.1	59.8 ± 5.8	52.5 ± 4.5	57.0 ± 5.8	
Urinary volume, *mL/d*					
Normal Ca, CO_3^{2-}	89.3 ± 18.0	91.9 ± 9.4	85.5 ± 9.0	68.8 ± 7.6	
Normal Ca, Cl^-	103.4 ± 8.1	95.3 ± 9.5	79.5 ± 10.6	74.6 ± 10.8	
High Ca, CO_3^{2-} + Cl^-	103.3 ± 14.2	103.6 ± 12.7	101.8 ± 13.3	113.2 ± 27.3	
Balance, *mmol/d*					
Calcium					
Intake					D,A
Normal Ca, CO_3^{2-}	9.7 ± 1.0^A	10.0 ± 1.1^A	8.4 ± 0.9^A	7.3 ± 1.0^A	
Normal Ca, Cl^-	11.0 ± 0.5^A	10.5 ± 0.6^A	8.5 ± 0.9^A	7.4 ± 0.8^A	
High Ca, CO_3^{2-} + Cl^-	19.8 ± 2.2^B	18.5 ± 1.8^B	16.1 ± 1.4^B	17.5 ± 1.8^B	
Urinary output					A
Normal Ca, CO_3^{2-}	0.09 ± 0.02	0.09 ± 0.02	0.08 ± 0.01	0.07 ± 0.02	
Normal Ca, Cl^-	0.12 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	
High Ca, CO_3^{2-} + Cl^-	0.10 ± 0.02	0.08 ± 0.02	0.06 ± 0.01	0.08 ± 0.01	
Fecal output					D,A
Normal Ca, CO_3^{2-}	5.9 ± 0.9^A	7.2 ± 0.9^A	7.7 ± 1.0^A	6.6 ± 0.8^A	
Normal Ca, Cl^-	6.1 ± 0.5^A	7.9 ± 0.6^A	6.3 ± 0.7^A	7.0 ± 0.8^A	
High Ca, CO_3^{2-} + Cl^-	15.4 ± 2.0^B	15.2 ± 1.5^B	15.0 ± 1.5^B	18.0 ± 1.9^B	
Retention					A
Normal Ca, CO_3^{2-}	3.7 ± 0.4	2.6 ± 0.4	0.7 ± 0.4	0.6 ± 0.4	
Normal Ca, Cl^-	4.8 ± 0.5	2.6 ± 0.3	2.2 ± 0.3	0.3 ± 0.5	
High Ca, CO_3^{2-} + Cl^-	4.3 ± 0.6	3.3 ± 0.6	1.0 ± 0.9	-0.6 ± 0.4	
Magnesium					
Intake					A
Normal Ca, CO_3^{2-}	0.80 ± 0.08	0.82 ± 0.09	0.71 ± 0.07	0.61 ± 0.08	
Normal Ca, Cl^-	0.92 ± 0.04	0.88 ± 0.05	0.71 ± 0.07	0.62 ± 0.07	
High Ca, CO_3^{2-} + Cl^-	0.92 ± 0.10	0.86 ± 0.08	0.80 ± 0.07	0.87 ± 0.09	
Urinary output					A
Normal Ca, CO_3^{2-}	0.23 ± 0.03^{A,B}	0.28 ± 0.03	0.28 ± 0.02	0.24 ± 0.03	
Normal Ca, Cl^-	0.32 ± 0.02^A	0.29 ± 0.03	0.29 ± 0.03	0.26 ± 0.03	
High Ca, CO_3^{2-} + Cl^-	0.20 ± 0.03^B	0.24 ± 0.03	0.25 ± 0.02	0.20 ± 0.02	
Fecal output					D,A
Normal Ca, CO_3^{2-}	0.35 ± 0.06^A	0.44 ± 0.05	0.50 ± 0.08	0.46 ± 0.06^A	
Normal Ca, Cl^-	0.46 ± 0.03^{A,B}	0.56 ± 0.04	0.44 ± 0.04	0.51 ± 0.04^A	
High Ca, CO_3^{2-} + Cl^-	0.59 ± 0.08^B	0.61 ± 0.06	0.56 ± 0.06	0.74 ± 0.26^B	
Retention					A
Normal Ca, CO_3^{2-}	0.21 ± 0.04	0.10 ± 0.04	-0.07 ± 0.04	0.15 ± 0.05	
Normal Ca, Cl^-	0.14 ± 0.01	0.04 ± 0.03	-0.01 ± 0.04	0.11 ± 0.06	
High Ca, CO_3^{2-} + Cl^-	0.14 ± 0.03	0.02 ± 0.06	-0.01 ± 0.04	0.13 ± 0.02	
Phosphorus					
Intake					A
Normal Ca, CO_3^{2-}	9.2 ± 1.0	9.5 ± 1.0	8.6 ± 0.9	7.4 ± 1.0	
Normal Ca, Cl^-	10.8 ± 0.5	10.4 ± 0.6	8.2 ± 0.8	7.2 ± 0.8	
High Ca, CO_3^{2-} + Cl^-	10.3 ± 1.1	9.6 ± 0.9	8.1 ± 0.7	8.8 ± 0.9	
Urinary output					D,A,D·A
Normal Ca, CO_3^{2-}	4.6 ± 0.5^A	5.8 ± 0.3^A	4.9 ± 0.3^A	4.4 ± 0.5	
Normal Ca, Cl^-	5.0 ± 0.4^A	4.8 ± 0.4^A	4.6 ± 0.5^A	4.5 ± 0.5	
High Ca, CO_3^{2-} + Cl^-	2.8 ± 0.6^B	3.1 ± 0.5^B	3.1 ± 0.3^B	3.5 ± 0.4	
Fecal output					D,A,D·A
Normal Ca, CO_3^{2-}	1.5 ± 0.4^A	2.1 ± 0.4^A	2.5 ± 0.5^A	2.4 ± 0.4^A	
Normal Ca, Cl^-	2.3 ± 0.2^A	3.5 ± 0.2^B	2.5 ± 0.2^A	2.9 ± 0.4^A	
High Ca, CO_3^{2-} + Cl^-	4.3 ± 0.6^B	5.0 ± 0.4^C	4.2 ± 0.4^B	5.5 ± 0.6^B	
Retention					A
Normal Ca, CO_3^{2-}	3.1 ± 0.3	1.7 ± 0.4	1.2 ± 0.2	0.7 ± 0.6	
Normal Ca, Cl^-	3.6 ± 0.2	2.1 ± 0.2	1.2 ± 0.2	-0.2 ± 0.3	
High Ca, CO_3^{2-} + Cl^-	3.2 ± 0.4	1.6 ± 0.5	0.8 ± 0.5	-0.2 ± 0.3	

[1]Values are means ± SEM (n = 6 or 8).

[2]Within a column, values not sharing the same superscript are significantly different (Tukey, $P < 0.05$).

[3]Significance: Multivariate ANOVA, repeated measures ($P < 0.05$); D = diet effect; A = age effect; D·A = interaction of diet and age.

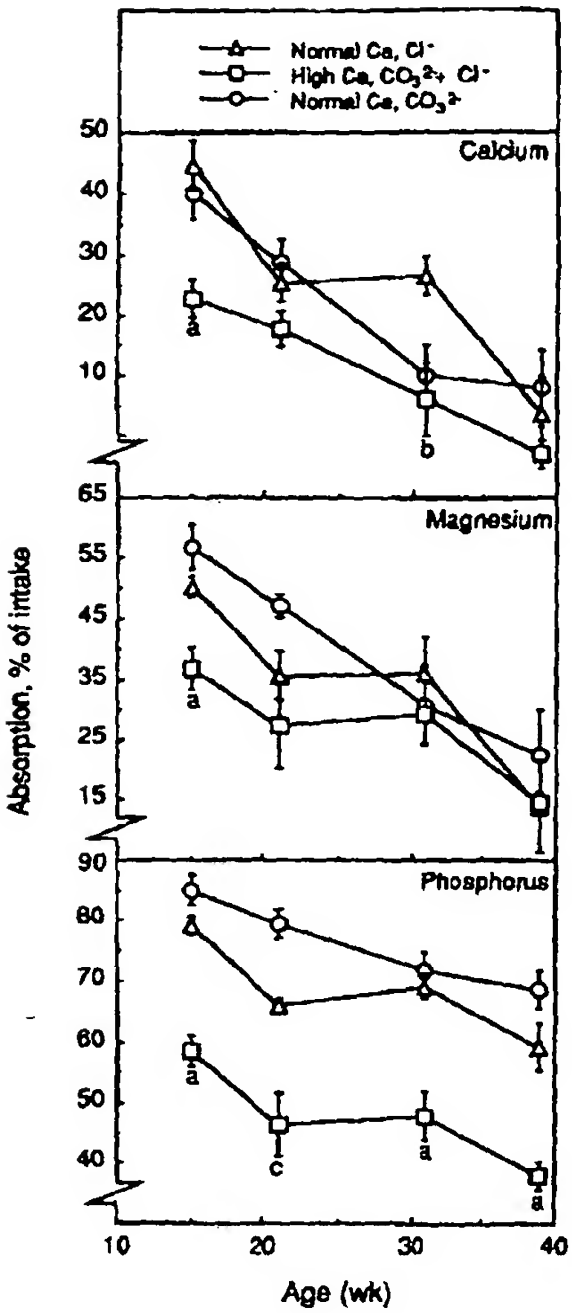

FIGURE 4 Experiment 2: Time course of the percentages of apparent absorption of calcium, magnesium and phosphorus in female kittens fed diets containing either normal levels of calcium with either calcium carbonate or calcium chloride or a high level of calcium with a combination of the two salts (Table 1). Results are expressed as means ± SEM (normal calcium diet with calcium carbonate: $n = 6$; normal and high calcium diet with calcium chloride: $n = 8$). Multivariate ANOVA, repeated measures ($P < 0.05$), revealed significant effects of diet and age on the percentages of apparent absorption of calcium, magnesium and phosphorus. One-way ANOVA was performed to compare the three dietary groups at the same age: calcium absorption: wk 15, $P < 0.001$; wk 31, $P < 0.05$; magnesium absorption: wk 15, $P < 0.001$; phosphorus absorption: wk 15, 21, 31 and 39, $P < 0.001$. The Tukey test ($P < 0.05$) revealed the following significant differences: *a*, high calcium diet vs. the two normal calcium diets; *b*, high calcium diet with calcium carbonate plus calcium chloride vs. normal calcium diet with calcium chloride; *c*, each diet vs. the other two diets.

calcium diet. Retention of calcium, magnesium and phosphorus were not affected by dietary composition, but dropped with age. Group mean retentions of magnesium were negative at the age of 31 to 39 wk.

Mineral absorption. Apparent absorptions of calcium, magnesium and phosphorus, expressed as percentage of intake, were generally lower in the kittens fed the high calcium diet (Fig. 4). In the kittens fed the high calcium diet magnesium absorption was significantly lower only at the age of 15 wk. Until the age of 21 wk apparent absorption of magnesium tended to be lower in the kittens fed the normal calcium diet with calcium chloride compared with the kittens fed the normal calcium diet with calcium carbonate. Also the absorption of phosphorus tended to be lower in kittens fed the normal calcium diet containing calcium chloride instead of calcium carbonate. Percentages of apparent absorption of calcium, magnesium and phosphorus dropped with age.

Urinary composition. Urinary volume was not significantly different in the three dietary groups, but tended to be greater in the kittens fed the high calcium diet (Table 3). Urinary pH was lowest in the kittens fed the normal calcium diet with calcium chloride, and decreased until the age of 31 wk (Fig. 5). Feeding the high calcium diet containing both calcium carbonate and calcium chloride produced intermediate urinary pH values. Urinary concentrations of calcium were not significantly affected by diet. Kittens fed the high calcium diets had lower urinary concentrations of magnesium and phosphorus. Urinary concentrations of phosphorus in the kittens fed the normal calcium diet with calcium chloride were lower than those in kittens fed the normal calcium diets with calcium carbonate.

Plasma minerals. Plasma concentrations of calcium and magnesium were similar for the three dietary groups and declined from values of 2.65 ± 0.05 and 0.86 ± 0.02 mmol/L at the age of 11 wk to 2.43 ± 0.02 and 0.82 ± 0.02 mmol/L at the age of 39 wk (means ± SEM, $n = 22$). Plasma levels of phosphorus were significantly ($P < 0.05$, MANOVA, repeated measures) higher in the cats fed the diets containing calcium chloride; overall group values were 2.01 ± 0.07 mmol/L for the normal calcium diet with calcium carbonate (means ± SEM, $n = 6$), 2.26 ± 0.03 mmol/L for the normal calcium diet with calcium chloride ($n = 8$) and 2.27 ± 0.05 mmol/L for the high calcium diet with both calcium carbonate and calcium chloride ($n = 8$). Plasma concentrations of phosphorus decreased from 2.25 ± 0.08 mmol/L at the age of 11 wk to 1.85 ± 0.05 mmol/L at the age of 39 wk (means ± SEM, $n = 22$).

Urea and creatinine concentrations. Plasma levels and urinary excretion of urea and creatinine were not significantly affected by dietary composition. From the age of 11 to 39 wk, plasma urea rose from 5.4 ± 0.3 to 6.8 ± 0.4 mmol/L and plasma creatinine from

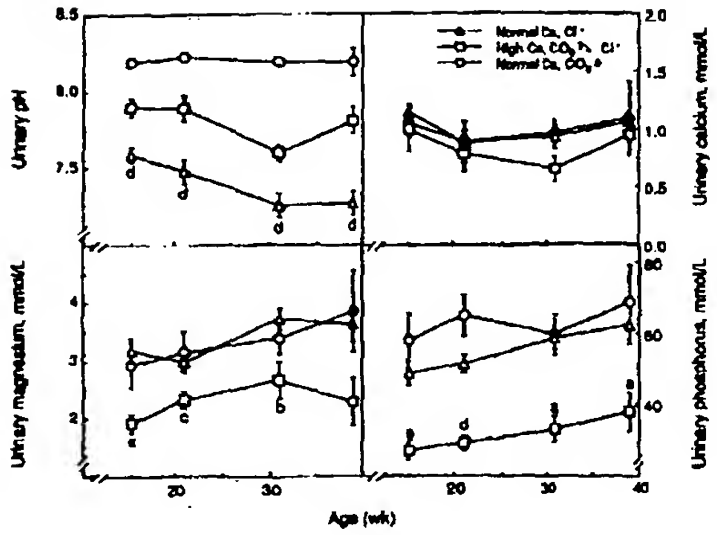

FIGURE 5 Experiment 2: Time course of urinary pH and urinary concentrations of calcium, magnesium and phosphorus in female kittens fed diets containing either normal levels of calcium with either calcium carbonate or calcium chloride or a high level of calcium with a combination of the two salts (Table 1). Results are expressed as means ± SEM (normal calcium diet with calcium carbonate: $n = 6$; normal and high calcium diet with calcium chloride: $n = 8$). Multivariate ANOVA, repeated measures ($P < 0.05$), revealed significant effects of diet and age on urinary pH and urinary concentrations of magnesium and phosphorus. Urinary pH was also affected by a diet-age interaction. Urinary concentration of calcium was affected by age. One-way ANOVA was performed to compare the three dietary groups at the same age: urinary pH: wk 15, 21, 31 and 39, $P < 0.001$; urinary magnesium: wk 15, $P < 0.01$; wk 21, 31 and 39, $P < 0.05$; urinary phosphorus: wk 15, 21 and 31, $P < 0.001$; wk 39, $P < 0.05$. The Tukey test ($P < 0.05$) revealed the following significant differences: *a*, high calcium diet vs. the two normal calcium diets; *b*, high calcium diet with calcium carbonate plus calcium chloride vs. normal calcium diet with calcium chloride; *c*, high calcium diet with calcium carbonate plus calcium chloride vs. normal calcium diet with calcium carbonate; *d*, each diet vs. the other two diets.

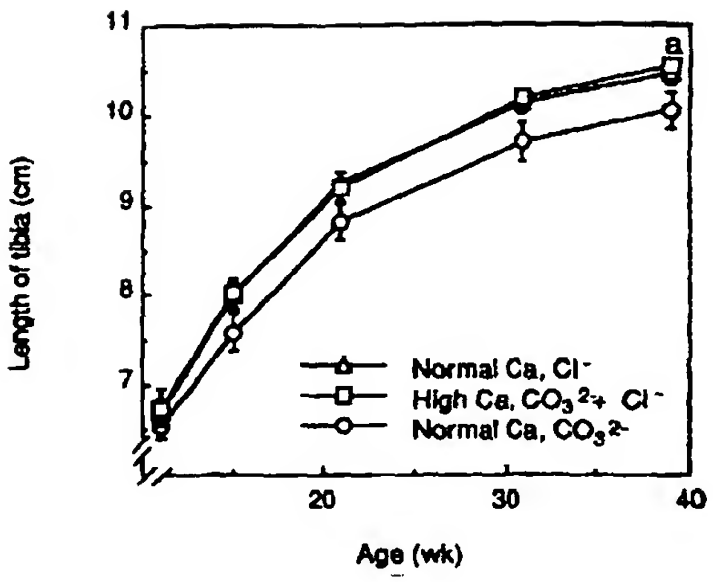

FIGURE 6 Experiment 2: Time course of length of tibia of female kittens fed diets containing either normal levels of calcium with either calcium carbonate or calcium chloride or a high level of calcium with a combination of the two salts (Table 1). Results are expressed as means ± SEM (normal calcium diet with calcium carbonate: $n = 6$; normal and high calcium diet with calcium chloride: $n = 8$). Multivariate ANOVA, repeated measures ($P < 0.05$), revealed a significant effect of age on length of tibia. One-way ANOVA was performed to compare the three dietary groups at the same age: wk 31 and 39, $P < 0.05$. The Tukey test ($P < 0.05$) revealed the following significant difference: *a*, high calcium diet with calcium carbonate plus calcium chloride vs. normal calcium diet with calcium carbonate.

58 ± 1 to 110 ± 5 μmol/L (means ± SEM, $n = 22$). Urinary urea excretion dropped from 19.7 ± 0.7 mmol/(d·kg body wt) at the age of 15 wk to 13.0 ± 0.6 mmol/(d·kg body wt) at the age of 39 wk, and urinary creatinine excretion rose from 319 ± 10 to 352 ± 10 μmol/(d·kg body wt) (means ± SEM, $n = 22$).

Bone development. Tibiae of the kittens fed either the normal calcium diet with calcium chloride or the high calcium diet with calcium carbonate plus calcium chloride were systematically, but not always significantly longer than those of the cats fed the normal calcium diet with calcium carbonate (Fig. 6). Plasma activity of alkaline phosphatase, which is an indicator of bone formation, and urinary excretion of hydroxyproline, which is an indicator of bone resorption, were not influenced by dietary composition, but decreased with age; values were 2.8 ± 0.1 μkat/L and 72 ± 4 μmol/(d·kg body wt) at the age of 15 wk and 1.0 ± 0.1 μkat/L and 19 ± 1 μmol/(d·kg body wt) at the age of 39 wk (means ± SEM, $n = 22$).

Dietary composition did not affect final (age 39 wk) length, circumference, dry weight, volume and ash content of femur; the values were 9.7 ± 0.1 cm, 2.5 ± 0.04 cm, 6.1 ± 0.2 g, 4.6 ± 0.1 cm^3, and 64.0 ± 0.2 g/100 g, respectively (means ± SEM, $n = 22$). In the cats fed the two diets with calcium chloride compared with those given the normal calcium diet with calcium carbonate, femur density was significantly greater (1.37 ± 0.02 and 1.35 ± 0.01 vs. 1.28 ± 0.02 g/cm^3, means ± SEM, $n = 6$ or 8, $P < 0.01$). Femur calcium concentration was higher in the cats fed the diets containing calcium chloride, when expressed as mmol/cm^3 (7.7 ± 0.1 and 7.7 ± 0.1 vs. 7.3 ± 0.1, means ± SEM, $n = 6$ or 8, $P < 0.05$), but not when expressed relative to dry weight. Femur magnesium and phosphorus concentrations were similar for all three dietary groups, the overall values being 0.19 ± 0.003 and 4.8 ± 0.1 mmol/cm^3, respectively (means ± SEM, $n = 22$).

Mineral concentrations of organs. Calcium concentrations in the hearts of the cats fed the two calcium chloride–containing diets were slightly lower

TABLE 4

Experiment 2: Mineral concentrations of kidneys in kittens fed normal calcium diets containing either calcium carbonate or calcium chloride or a high calcium diet with calcium carbonate plus calcium chloride[1,2]

	Normal Ca		High Ca
	CO_3^{2-}	Cl^-	CO_3^{2-} + Cl^-
Kidney mineral content	μmol/g dry weight		
Calcium	12 ± 2[B]	7 ± 0.3[A]	7 ± 0.1[A]
Magnesium	26 ± 0.8	24 ± 0.4	24 ± 0.6
Phosphorus	367 ± 10	356 ± 6	354 ± 8

[1]Results are expressed as means ± SEM (*n* = 6 or 8).

[2]One-way ANOVA or Kruskal-Wallis test was performed to compare the three dietary groups: kidney calcium content: $P < 0.001$. Groups not sharing the same superscript are significantly different (Mann-Whitney U test, $P < 0.017$).

when compared with those of cats fed the normal calcium diet with calcium carbonate (2.9 ± 0.1 and 3.0 ± 0.1 vs. 3.4 ± 0.2, means ± SEM, $n = 6$ or 8, $P < 0.05$), but the concentrations of magnesium and phosphorus were unaffected by dietary treatment, the pooled values being 35 ± 0.4 and 287 ± 2 μmol/g dry wt (means ± SEM, $n = 22$). Calcium, magnesium and phosphorus concentrations in liver were similar for all three dietary groups; the overall values were 2.7 ± 0.1, 23 ± 0.4 and 295 ± 6 μmol/g dry wt, respectively (means ± SEM, $n = 22$). Kidney calcium was significantly lower in the cats fed the diets with calcium chloride (Table 4). The three dietary groups did not differ with regard to renal concentrations of magnesium and phosphorus.

DISCUSSION

In both the adult and young cats, urinary pH was lower when dietary calcium chloride was substituted for calcium carbonate or when calcium chloride was added to the normal calcium diet with calcium carbonate. The acidifying effect of calcium chloride relates to the fact that calcium is much less well absorbed than chloride. Urinary excretion of absorbed chloride will lower the urinary excretion of bicarbonate, raise that of ammonium and lower urinary pH. In the adult cats, the addition of calcium carbonate to the normal calcium diet containing calcium chloride raised urinary pH, but it did not do so after addition to the normal calcium diet containing calcium carbonate, which already yielded a relatively high urinary pH. As would be anticipated, the supplemental calcium carbonate systematically lowered urinary excretion of titratable acid and net acid excretion.

The substitution of dietary calcium chloride for calcium carbonate reduced urinary concentrations of calcium and phosphorus in the adult cats. Likewise, the kittens fed the low calcium diet with calcium chloride tended to have lower urinary phosphorus concentrations than their counterparts fed the low calcium diet with calcium carbonate. The finding that dietary calcium chloride as compared with calcium carbonate lowered urinary calcium and phosphorus in the cats is quite unexpected. A reduction in urinary pH as induced by the feeding of ammonium chloride or replacement of dietary calcium carbonate by calcium chloride, is associated with a rise in urinary excretion of calcium in rats (Kunkel et al. 1986, Schaafsma et al. 1985, Whiting and Cole 1986). Urinary acidification in rats may also produce a rise in urinary excretion of phosphorus (Greger et al. 1991, Kraut et al. 1986). In cats, urinary acidification as induced by ammonium chloride elevated urinary calcium excretion in the experiment of Ching et al. (1989), but not in that of Dow et al. (1990). The observed discrepancy with respect to urinary concentrations of calcium and phosphorus may relate to the fact that the urinary pH after feeding calcium chloride (Fig. 2 and 5) did not reach values as low (pH 5.7–6.1) as those seen in the other studies (Ching et al. 1989).

We can only speculate as to the mechanism underlying the lower urinary concentration of phosphorus in cats fed diets with calcium chloride. Possibly, the urinary concentration of bicarbonate is involved; this concentration is low at low pH, leading to enhanced tubular phosphorus reabsorption (Kuntziger 1980). The lower urinary pH in the cats given calcium chloride also induces a shift from HPO_4^{2-} to $H_2PO_4^-$, the latter possibly being more readily reabsorbed by the renal tubules (Mercado et al. 1975), but the opposite has also been suggested (Mizgala and Quamme 1985).

Urinary concentrations of minerals were also affected by the level of dietary calcium. Cats fed high calcium diets had a lower absorption of magnesium and phosphorus, which was reflected by lower urinary concentrations of these minerals. High calcium intakes have been shown to enhance the formation of an insoluble calcium-magnesium-phosphate complex in the intestine, and thus limit the availability of magnesium and phosphorus for absorption (Brink et al. 1992).

Calcium chloride vs. calcium carbonate in the normal calcium diet of the kittens tended to stimulate body weight gain and tibia growth. These effects were associated with a higher femur density and a higher femur calcium content. The addition of chloride to the diet in the form of ammonium chloride caused a negative calcium balance in cats (Ching et al. 1989), which may be associated with loss of bone minerals as shown in rats (Kraut et al. 1986, Kunkel et al. 1986). Clearly, we did not find such an

adverse effect. The explanation may lie in the fact that we enriched the diets with calcium chloride instead of ammonium chloride and/or that urinary pH values did not fall below 7.

The development of nephrocalcinosis, as based on kidney calcium contents, was inhibited in the kittens fed diets containing calcium chloride. Probably, the lower values of urinary pH prevented renal calcification as demonstrated in rats (Kootstra et al. 1991). High degrees of nephrocalcinosis may impair kidney function in rats (Ritskes-Hoitinga et al. 1989). Renal function in the cats with elevated levels of kidney calcium was not impaired as based on plasma concentrations of urea and creatinine and creatinine clearance. Plasma levels of creatinine were relatively high, but were within the normal range for cats (Melby and Altman 1974). The high creatinine levels may relate to the presence of fish meal in the purified diets, which raises plasma creatinine concentrations in rats (Zhang and Beynen 1992). Lucke and Hunt (1967) observed a high incidence of nephrocalcinosis in domestic cats, which may eventually impair renal function (Lewis et al. 1987). The incorporation of calcium chloride into the diet may be useful in the reduction of the kidney calcium content in cats.

Urinary pH and urinary phosphorus excretion were lower in cats and kittens fed diets with calcium chloride instead of calcium carbonate. Body weight gain, tibia growth and femur density in the kittens fed diets with calcium chloride were higher while renal calcium level was lower than in their counterparts fed the normal calcium diet with calcium carbonate.

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